

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title: Validation of sperm fertility biomarkers in the rabbit
 LAPR Number: 19-03-002
 Principal Investigator: **Exemption 6**
 Author of this Document: **Exemption 6**/RTP/USEPA/US
 Date Originated: 03/11/2016
 LAPR Expiration Date: 03/31/2019
 Agenda Date: 03/23/2016
 Date Approved: 03/29/2016
 Date Closed:

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US	03/29/2016	DMR	
	by Exemption 6 /RTP/USEPA/US Exemption 6 Exemption 6 /RTP/USEPA/US	03/29/2016	DMR	

persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

The objectives of this LAPR are twofold: 1) To use fresh ejaculated rabbit sperm to validate the use of specific antibodies to a sperm protein (SP22) and potentially other biomarkers of sperm quality; 2) To expose sperm to environmental chemicals to determine if they perturb sperm function in vitro.

The rabbit serves as a good model to study ejaculated sperm quality as it is impossible to obtain ejaculated sperm from rodents and the mission in my laboratory is to relate chemical induced alterations in sperm quality to human reproductive health risk.

In the previous rabbit LAPRs we were able to confirm that a 22 amino acid peptide in the SP22 protein was indeed localized to the equatorial segment, and more importantly that the localization observed was dependent on the viability of the sperm in the ejaculate. Immunostaining of sperm devoid of viability dye (live sperm) is restricted to the equatorial segment, whereas damaged sperm show atypical SP22 localization and dead sperm fail to recognize the SP22 antibody (see attached). We have only begun to look at probes to quantitate oxidative damage to the rabbit sperm membrane and part of a battery of assessments (SP22, viability, oxidative damage, motility) following in vitro chemical insult.

1) In previous LAPRs we identified an exposed and functional region of the fertility biomarker SP22. We are now generating a monoclonal antibody to this region using the human sequence. We are relying heavily on how this antibody will immunostain ejaculated rabbit sperm since the rabbit sperm morphology is very similar to that of humans sperm. Once characterized, the new antibody will be used in a human study in Brazil. Hopefully data from this study will bolster consideration of this biomarker for use in a Diagnostic Kit of sperm quality in men and EPA will be able to license the technology. We will be using ejaculated rabbit sperm to determine that the localization and level of immunostaining with this new antibody does relate to sperm quality as evaluated using viability dyes and markers of oxidative damage. Like humans, sperm quality in the ejaculate of the rabbit varies significantly among individual males and from collection to collection for a given male. This inherent individual variation in sperm quality between ejaculates will also help us establish a threshold amount of SP22 for sperm with sufficient motility and viability.

2) Finally, given the growing emphasis towards in vitro screening we realize the value of using sperm from rabbit ejaculates to screen for the potential of environmental chemicals to perturb human sperm function directly. The expression of membrane proteins like SP22, viability, oxidative damage, and sperm motility can be quantified over time upon exposure of ejaculated sperm to increasing concentrations of test environmentally relevant chemicals.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

There is no in vitro system for the production of sperm.

b. Justify the species requested:

The rabbit is the preferred laboratory model to study ejaculated sperm quality as it is impossible to obtain ejaculated sperm from rodents.

3. How was it determined that this study is not unnecessary duplication?

A search of PubMed, Medline shows that this research is not duplication of effort. No one is conducting research to evaluate qualitative differences in ejaculated rabbit sperm based on biomarker immunostaining. Additionally, no one is using ejaculated rabbit sperm to screen for the potential of priority chemicals to alter sperm quality directly.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include

critical information within the body of the LAPR.

We need to collect sperm from rabbits to conduct in vitro studies (1 or 2 above). Ejaculates are collected from each of 3-4 males. Typically, no more than 2 ejaculates are collected in a week. Often if the rabbit has not been collected for some time (months), the first ejaculate contains a lot of cellular debris and the rabbit is collected again the next day. There is no pre-determined number of ejaculates collected per rabbit per year. We can use any of the females as teasers on ejaculate collection days.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

We need to keep at least 6 males producing ejaculates at all times. We have found that there is sufficient variability both between males and between ejaculates for a given male. With 4 males producing ejaculates, we can get at least 2 usable ejaculates per collection. We need to have a group of at least 4 females to use as teasers.

Although rare, there is the possibility of an accidental mating that could result in the birth of unintended offspring. Accordingly, we include offspring to cover one such mating; we've only encountered this once in over 20 years.

We currently have 10 rabbits (4 female and 6 male rabbits) to be transferred from the expiring LAPR. Historically, we replace six animals over the course of 3 yrs due to old age, hence the request to order six additional animals.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	16	8
D) Potential pain/distress relieved by appropriate measures:	0	0
E) Unrelieved pain/distress:	0	0

4. Does this LAPR include any of the following:

- ☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Typically, no more than 2 ejaculates are collected in a week. Collection of ejaculates is done using a pre-fabricated, water jacketed artificial vagina and a teaser doe. The water in the water jacket does not exceed 40 degrees C and a small volume of glycerol is added to the latex opening to facilitate entry. The doe is brought into the cage with the male, and the artificial vagina is introduced to the penis at the time of mounting.

b. Survival Blood Collections (method, volume, frequency):

N/A

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

N/A

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

N/A

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

N/A

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:

N/A

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

N/A

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

N/A

c. Testing methods:

N/A

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

N/A

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

N/A

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

N/A

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

N/A

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

N/A

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

N/A

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

N/A

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

N/A

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

☐ Yes ☒ No

f. Identify any surgical procedures performed at other institutions or by vendors:

N/A

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

none

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

The animals are monitored once a week by laboratory staff. If an animal begins to lose weight or develop a detectable lesion or mass the Attending Veterinarian is contacted. Common health issues that may occur in

rabbits include: adenocarcinoma, bladder sludge, gastric stasis (hair ball). Bladder sludge and gastric stasis can be treated adequately with fluids and analgesics, but adenocarcinoma requires surgery, and if the animal is nearing the end of its laboratory usefulness, the recommendation would be to euthanize. Regardless of the disorder, if recommended treatments fail, and the animal's quality of life appears compromised, the animal will be euthanized.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

N/A

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	6
b. Animals to be transferred from another LAPR:	10
LAPR Number that is the source of this transfer:	16-03-002
c. Animals to be transferred from another source:	0
d. Offspring produced onsite (used for data collection and/or weaned):	8
e. TOTAL NUMBER of animals for duration of the LAPR	24

2. Species (limited to one per LAPR): Rabbit(s)

3. Strain: New Zealand White

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

N/A

4. Sources of animals:
Charles River Laboratory

5. Provide room numbers where various procedures will be performed on animals:

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

Exempt
Exempt
Exempt

Room Numbers:

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

N/A

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

N/A

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

N/A

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

New cages for housing rabbits in pairs are now in place. They have no wire bottom but have smooth rounded holes. Environmental enrichment includes regular exercise in an exercise area, they are given frequent baths and they have their geometric toys (rings, balls etc.,) to play with. Females will be socially housed in compatible pairs. Males have weekly "play-time" in pairs in the playpen area. During this "play-time" they are monitored. Because males do exhibit some mild aggressive behavior such as mounting one another they are not housed socially. Finally, new food items such as hay, vegetables, and fresh greens are provided as supplemental enrichment.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used. Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

N/A

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

N/A

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

N/A

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

N/A

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

N/A

Hint: The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	Study Design	Over 30 years of experience with rabbits. Initial training was obtained Exemption 6 on handling and surgery of laboratory and domestic animals. All NHEERL-required training is completed.
Exemption 6	Technical Staff	Collection of Ejaculates	Over 20 years of experience handling rabbits. All NHEERL-required training is completed.
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

- 1. Estimated number of breeding pairs and liveborn per year*** N/A
- 2. Breeding protocols and recordkeeping*** N/A
- 3. Methods for monitoring genetic stability*** N/A
- 4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR*** N/A

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Animals will be euthanized when ejaculate quality or antibody production declines, or if they have a health issue that can't be managed.

2. Describe the euthanasia techniques:

Method(s): Anesthesia
Agent(s): Pentobarbital (195 mg/ml solution)
Dose (mg/kg): 150 to 300 mg/kg
Volume: 1.6 ml/kg or to effect
Route: Intravenous or intraperitoneal

Source(s) of information used to select the above agents/methods:

2013 AVMA Guidelines on Euthanasia.
Veterinary Staff

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing

more than 200 grams).

N/A

4. Describe how death is to be confirmed.

Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Euthanized as above

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

☐ Yes ☒ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.

2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.

3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.

4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.

5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.

6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.

7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.

8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	03/11/2016

Submitted: 03/14/2016

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	Approval Date	Phone Number	Division	Mail Drop
Exemption 6	03/15/2016	Exemption 6 Lotus Notes Address	TAD Branch	MD Submitted to Branch Chief for Approval
	by Exemption 6 Exemption 6 Exemption 6	Exemption 6 Exemption 6 Exemption 6	RTB	03/14/2016 11:40 AM

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ATTACHMENTS



19-03-002 PI Resp.pdf



RabbitSpermSP22.pptx

Actions

First Update notification sent: 01/31/2017
Second Update notification sent: 03/02/2017
First 2nd Annual notification sent:
02/02/2018
Second 2nd Annual notification sent:
03/02/2018
1st Expiration notification sent:
2nd Expiration notification sent:

History Log: